

Exhibit A:***Sequences of 1A7***

The polynucleotide sequences were obtained for the 1A7 antibody by isolating messenger RNA from the 1A7 producing cell line. For each sequence determination, total RNA was isolated from $\sim 1 \times 10^6$ 1A7 hybridoma cells. The yield of total RNA was about 100 μ g. First strand cDNA was synthesized using SuperScript Preamplification kit (GIBCO/BRL).

To sequence the heavy chain variable region, PCRs were conducted on the cDNA using a reverse primer corresponding to amino acids 126 to 119 of the murine γ_1 constant region:

5'-CCCAAGCTTCCAGGGRCCARKGGATARACIGRTGG-3'

and various mixtures of forward primers, corresponding to the N-terminal leader sequences of murine variable region subgroups. The forward primer that gave a positive reaction was:

5'-ACTAGTCGACATGGCTGTCYTRGBGCTGYTCYTCTG-3'

corresponding to amino acids -20 to -12.

The amplified fragment of cDNA was subcloned into pT7 plasmid and NovaBlue competent cells were transformed using a protocol provided by the supplier (Novagen). Recombinant colonies were picked up by color selection and plasmid DNA

was prepared by miniprep procedure. The DNA sequence of the double stranded plasmid was determined using a Sequenase Version 2.0 kit (USB, Cleveland, Ohio). The sequence of the DNA insert in the plasmid was determined from both orientations using primers specific for the plasmid; namely T7 promoter (TAATACGACTCACTATAGGG) and U-19 (GTTTTCCCAGTCACGACGT). At least 8 clones were picked for sequence determination.

The sequence of the 1A7 light chain variable region was determined in a similar fashion. The forward and reverse primers giving a positive result in the PCR were:

5'-ACTAGTCGACATGAAGTTGCCTGTTAGGCTGTTGGTGCT-3'

5'-CCCAAGCTTACTGGATGGTGGGAAGATGGA-3'

corresponding to amino acids -19 to -10 of the leader sequence, and 122 to 116 of the mouse κ chain constant region.

The nucleic acid sequence and the corresponding translation for the light and heavy chain variable regions of monoclonal antibody 1A7 (along with neighboring residues of the leader and constant regions) are as follows:

1A7 light chain sequence

M K L P V R L L V L M F W I P A
ATG AAG TTG CCT GTT AGG CTG TTG GTG CTG ATG TTC TGG ATT CCT GCT
S S D
TCC AGC GAT (-1 to -19, leader)

D V L M T Q T P L S L P V S L G
GAT GTT TTG ATG ACC CAA ACT CCA CTC TCC CTG CCT GTC AGT CTT GGA
D Q A S I S C
GAT CAA GCC TCC ATC TCT TGC (1-23, Frame work 1)

R S S Q S I V H S N G N T Y L E
AGA TCT AGT CAG AGC ATT GTA CAT AGT AAT GGA AAC ACC TAT TTA GAA
(24-39, CDR 1)

W Y L Q K P G Q S P N L L I Y
TGG TAC CTA CAG AAA CCA GGC CAG TCT CCA AAC CTC CTG ATC TAC
(40-54, Frame work 2)

F V S N R F S
TTT GTT TCC AAC CGA TTT TCT (55-61, CDR 2)

G V P D R F S G S G S G T D F T
GGG GTC CCA GAC AGG TTC AGT GGC AGT GGA TCA GGG ACA GAT TTC ACA
L K I S R V E A E D L G V Y Y C
CTC AAG ATC AGC AGA GTG GAG GCT GAG GAT CTG GGA GTT TAT TAC TGC
(62-93, Frame work 3)

F Q G S H V P W T
TTT CAA GGT TCA CAT GTT CCG TGG ACG
(94-102, CDR 3)

F G G G T K L E I K
TTC GGT GGA GGC ACC AAG CTG GAA ATC AAA
(103-112, Frame work 4)

R A D A A P T V S I F P P
CGG GCT GAT GCT GCA CCA ACT GTA TCC ATC TTC CCA CCA

S S K L G
TCC AGT AAG CTT GGG (Constant region)

1A7 heavy chain sequence

M A V L G L L F C L V T F P S C
 ATG GCT GTC TTG GGG CTG CTC TTC TGC CTG GTG ACA TTC CCA AGC TGT
 V L S
 GTC CTG TCC (-1 to -19, Leader)

Q V Q V K E S G P F L V P P S Q
 CAG GTG CAG GTG AAG GAG TCA GGA CCT TTC CTG GTG CCC CCC TCA CAG
 S L S I T C T V S G F S L T
 AGC CTG TCC ATC ACA TGC ACT GTC TCA GGG TTC TCA TTA ACC
 (1-30, Frame work 1)

T Y G V S
 ACC TAT GGT GTA AGC (31-35, CDR 1)

W I R Q P P G K G L E W L G
 TGG ATT CGC CAG CCT CCA GGA AAG GGT CTG GAG TGG CTG GGA
 (36-49, Frame work 2)

A I W G D G T T N Y H S A L I S
 GCA ATT TGG GGT GAC GGG ACC ACA AAT TAT CAT TCA GCT CTC ATA TCC
 (50-65, CDR 2)

R L S I S K D N S K S Q V F L K
 AGA CTG AGC ATC AGC AAG GAT AAC TCC AAG AGC CAA GTT TTC TTA AAA
 L N S L Q T D D T A T Y Y C A K
 CTG AAC AGT CTG CAA ACT GAT GAC ACG GCC ACG TAC TAC TGT GCC AAA
 (66-97, Frame work 3)

L G N Y D A L D W
 CTG GGT AAC TAC GAT GCT CTG GAC TAC
 (98-106, CDR 3)

W G Q G T S V T V S S
 TGG GGT CAA GGA ACC TCA GTC ACC GTC TCC TCA
 (107-117, Frame work 4)

A K T T P P P V Y P L V P G S L
 GCC AAA ACG ACA CCC CCA CCC GTC TAT CCA TTG GTC CCT GGA AGC TTG GG
 (Constant region)